0022-202X/84/8201-0028$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 82:28–29, 1984

Vol. 82, No. 1
Printed in U.S.A.

Sequential Production of Birbeck Granules Through Adsorptive Pinocytosis

MASAMITSU ISHII, M.D., YU'ICHI TERAO, M.D., JUN-ICHI KITAJIMA, M.D., AND TOSHIO HAMADA, M.D.

Department of Dermatology, Osaka City University Medical School, Osaka, Japan

A case of vitiligo with inflammatory raised borders was observed electron microsocopically, resulting in an interesting view of the formation of Birbeck granules in Langerhans cells. Following the formation of larger coated vesicles, which perform adsorptive pinocytosis from the cell membrane, membrane invagination of the cell occurred shown as the tubular infolding, resulting in the observation of the characteristic Birbeck granule band pattern in its interior. This phenomenon supports the theory of Hashimoto and Tarnowski (1968) that Birbeck granules are formed from the infolding of the cell membrane. In addition, our study shows the involvement of adsorptive pinocytosis in the formation of the granules. It was suggested that when the coated Birbeck granule shifts into the cell, possibly its coat is detached and the vesicle portion forms the globule of the Birbeck granule.

In recent years, there is increasing evidence that a specific chain mechanism, referred to as adsorptive pinocytosis [1], occurs when substances such as low-density lipoprotein, ovovitellin, haptoglobin, transferrin, insulin, epidermal cell growth factor, triiodothyronine, lysosome enzyme, α_2-macroglobulin, etc. are absorbed into the cell [2–4].

In adsorptive pinocytosis, coated vesicles are torn off and carried within the cell as a reaction to the bonding between the substance taken in and a specific receptor at the cell surface [5,6].

This report examines a correlation suggested by the formation process of numerous Birbeck granules and coated vesicles found to be simultaneously present in many Langerhans cells (LC) whose characteristic form and function in vitiligo with inflammatory raised borders have previously been reported through electron microscopic observation [7].

PATIENT AND METHODS

The following is a brief summary of the patient and methods of the experiment, both presented in a previous report [7].

A 41-year-old female developed vitiligo vulgaris on the upper back and side of the neck 6 months prior to the initial examination. With the gradual spread of the vitiligo, erythematous borders developed in the area surrounding the vitiligo vulgaris on the side of the neck. In this infiltrated erythema, reddish papules, 1–2 mm in diameter were scattered.

A punch biopsy specimen was obtained from the erythematous border and prepared for electron microscopy. The specimen was fixed in 2.5% phosphate-buffered glutaraldehyde, postfixed in 2% phosphate-buffered osmic acid at pH 7.4, dehydrated in graded ethanol, and embedded in Epon 812. Thin sections were doubly stained with uranyl acetate and lead citrate and examined in a Hitachi HS-9 electron microscope.

Manuscript received June 14, 1983; accepted for publication August 26, 1983.

Reprint requests to: Masamitsu Ishii, M.D., Department of Dermatology, Osaka City University Medical School, 1-5-7, Asahi-machi, Abeno-ku, Osaka 545, Japan.

Abbreviations:
LC: Langerhans cell(s)

RESULTS

In a large number of LC, invagination of the tubular infolding of the cell membrane and connection of Birbeck granules to cell membrane were observed. Many invaginations, connections (Fig 1), and Birbeck granules that would have connected if serial section had been performed (Fig 1, *3 and *5) were simultaneously observed within 1 cross-section of the LC membrane.

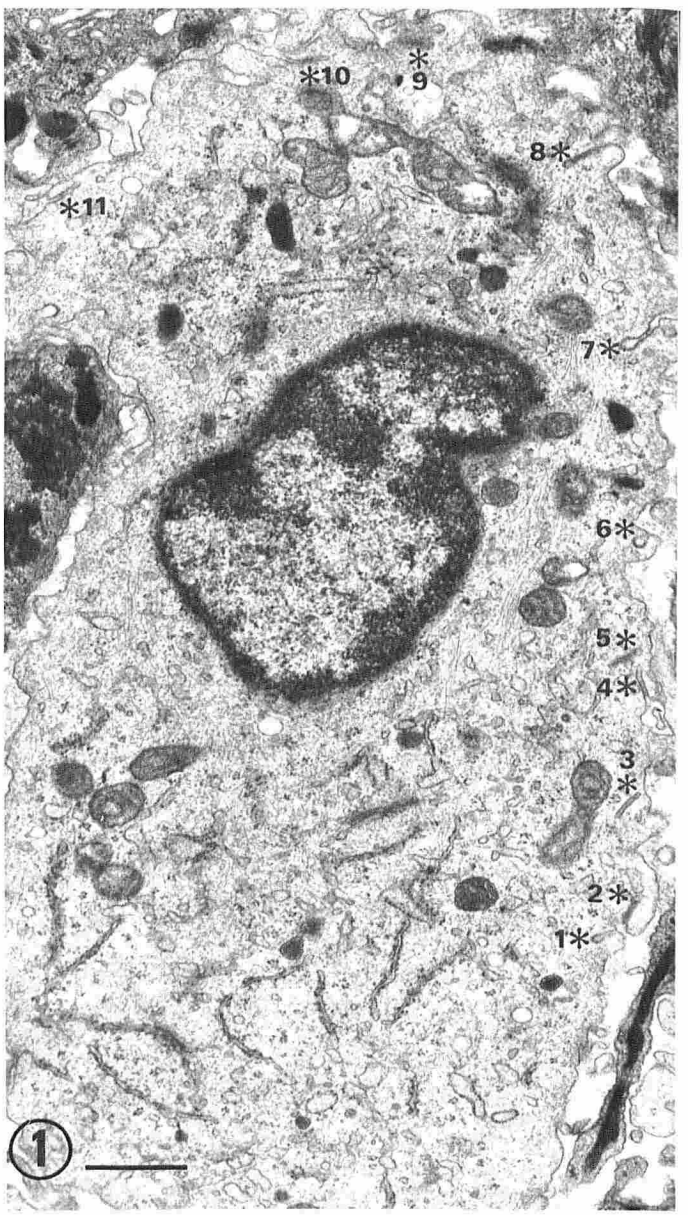

FIG 1. A Langerhans cell. Many connections of Birbeck granules to cytomembrane or invagination of cytomembrane are seen (*1–*11). *Bar* = 1 μm.

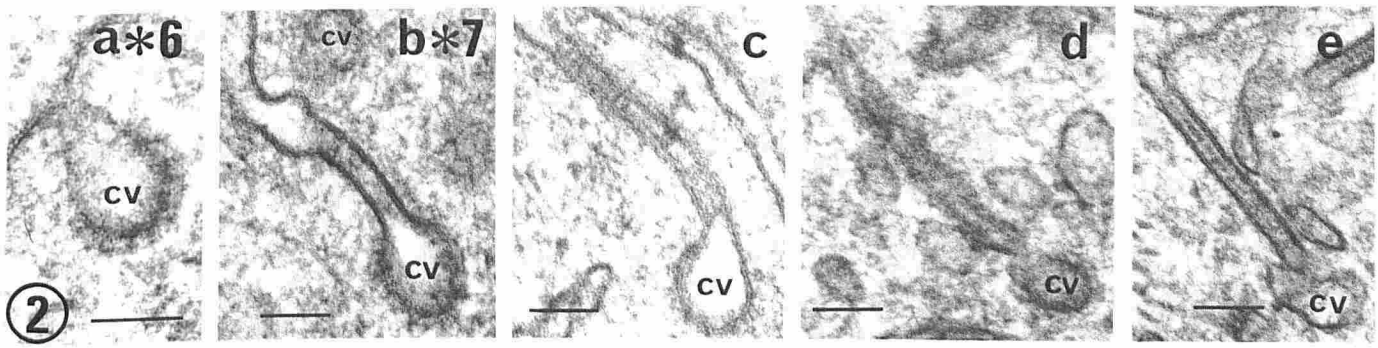

FIG 2. Enlargement of the areas marked *6 and *7 in Fig 1 (*a*6, b*7*) and parts of another Langerhans cell (*c, d, e*). *a*6*, Coated vesicles (cv) are formed on the cell membrane. *Bar* = 0.1 μm. *b*7*, Cell membrane connected to cv is invaginating. Free cv is also seen. *Bar* = 0.1 μm. *c*, Invagination becomes full length and a typical band pattern of Birbeck granule is formed. It looks like a Birbeck granule with a coat. *Bar* = 0.1 μm. *d*, Typical band pattern is obscure as obliquely sectioned, and cv is separating from the rod part. *Bar* = 0.1 μm. *e*, cv, which is transforming or shrinking, is seen. *Bar* = 0.1 μm.

From this observation invagination and connections of granules produced throughout the whole LC membrane were found to be extremely numerous. Coated vesicles were often found near the LC membrane (Fig 2, b*7) and also on the LC membrane (Fig 2, a*6 and b*7). However, coated vesicles formed on the LC membrane were often linked with invagination of the tubular infolding or connected Birbeck granules. Although these figures were seen simultaneously in one thin section of the cells, time lapse sequence of these phenomena could be guessed from their characteristics. The time lapse sequence guessed was as follows and it seemed to suggest the formative process of Birbeck granule.

1. Coated vesicles are formed on the cell membrane (Fig 2, a*6).
2. Invagination forms the tubular infolding of the cell membrane which is linked to the coated vesicle (Fig 2, b*7).
3. The cell membrane invagination grows to full length while a characteristic band pattern of Birbeck granule forms within the tubular part (Fig 2, c).
4. It was further observed that in some instances, the coated vesicle portion separates from the Birbeck granules (Fig 2, d) or is altered in appearance (Fig 2, e).

DISCUSSION

The origin of Birbeck granules has been disputed for some time [8]. By using lanthanum and peroxidase as tracers, Hashimoto et al [9–11] demonstrated that Birbeck granules are formed from the LC surface following the infolding of the LC membrane.

According to Wolff [12] and Niebauer et al [13], the granules derive from the Golgi apparatus. The granules move to the cell vicinity, adhering to the cell membrane and expelling the contents outside the cell. Recently,Takahashi and Hashimoto [14] reported new support for the cytomembrane origin of LC granules using an OKT-6 monoclonal antibody immunoperoxidase method on the cultured LC. Our results also support the theory of endocytotic origin, which is explained as follows. The coat of the larger coated vesicles, the appearance of which resembles a basket composed of pentagons and hexagons [15,16], is formed by the subtle operation of a protein substance called clathrin [17]. The cell membrane invagination takes on the characteristics of a Birbeck granule before it is separated from the cell membrane. Although this phenomenon is not observed in all Birbeck granules, adherence to cell membrane and membrane invagination, even if partially detected, clearly indicates that Birbeck granules are formed by cell membrane invagination.

In some cases, the coated vesicle separates from the Birbeck granule or is altered in appearance, therefore it is not certain whether the coated vesicle portion of the Birbeck granules will form the bulb part of the granule or not.

In the present study, we revealed the involvement of adsorptive pinocytosis [1] in the formation processes of Birbeck granules. Since adsorptive pinocytosis is the mechanism by which specific macromolecules and particular membrane components are selectively internalized by the cells [2], an interesting subject for future study is the type of substances that are selectively taken into Birbeck granules.

REFERENCES

1. Silverstein SC, Steinman RM, Cohn ZA: Endocytosis. Annu Rev Biochem 46:669–722, 1977
2. Goldstein JL, Anderson RGW, Brown MS: Coated pits, coated vesicles, and receptor-mediated endocytosis. Nature 279:679–685, 1979
3. Pastan IH, Willingham MC: Journey to the center of the cell: role of the receptosome. Science 214:504–509, 1981
4. Ashwell C, Harford J: Carbohydrate-specific receptors of the liver, Annu Rev Biochem 51:531–554, 1982
5. Mckanna JA, Haigler HT, Cohen S: Hormone receptor topology and dynamics: morphological analysis using ferritin-labeled epidermal growth factor. Proc Natl Acad Sci USA. 76:5689–5693, 1979
6. Pearse BMF: Coated vesicles. Trends Biochem Sci 5:131–134, 1980
7. Ishii M, Hamada T: Ultrastructural studies of vitiligo with inflammatory raised borders. J Dermatol (Tokyo) 8:313–322, 1981
8. Lever WF, Schaumburg-Lever G: Histopathology of the Skin, 6th ed. Philadelphia, JB Lippincott, 1983
9. Hashimoto K, Tarnowski WM: Some new aspects of the Langerhans cell. Arch Dermatol 97:450–464, 1968
10. Hashimoto K: Lanthanum staining of Langerhans' cell. Communication of Langerhans' cell granules with extracellular space. Arch Dermatol 102:280–290, 1970
11. Hashimoto K: Langerhans cell granule. An endocytotic organelle. Arch Dermatol 104:148–160, 1971
12. Wolff K: The fine structure of the Langerhans cell granule. J Cell Biol 35:468–477, 1967
13. Niebauer G, Krawezyk WS, Kidd RL, Wilgram GF: Osmium zinc iodide reactive sites in the epidermal Langerhans cell. J Cell Biol 43:80–89, 1969
14. Takahashi S, Hashimoto K: Derivation of Langerhans cell granules from cytomembrane (abstr). J Invest Dermatol 80:345, 1983
15. Kanaseki T, Kadota K: The "vesicle in a basket." A morphological study of the coated vesicle isolated from the nerve endings of the guinea pig brain, with special reference to the mechanism of membrane movements. J Cell Biol 42:202–220, 1969
16. Heuser J, Evans L: Three-dimensional visualization of coated vesicle formation in fibroblasts. J Cell Biol 84:560–583, 1980
17. Crowther RA, Pearse BMF: Assembly and packing of clathrin into coats. J Cell Biol 91:790–797, 1981